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EFFECTS OF CONTAMINANTS ON TELEOST REPRODUCTION: PAST AND ONGOING STUDIES

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1. Introduction

Aquatic organisms chronically exposed to low levels of environmental pollutants may eventually suffer serious physiological damage. This damage could result in the impairment of the reproductive processes of the affected organisms. Resource managers are concerned that commercially important fish species inhabiting polluted areas may be undergoing such impairment. Reduced regeneration or recruitment of these fish could lead to the eventual decline of their populations. This potential threat to natural resources has encouraged the Ocean Assessments Division, Office of Oceanography and Marine Assessment, National Ocean Service of the National Oceanic and Atmospheric Administration to sponsor projects aimed at identifying and monitoring the effects of contaminants on fish reproduction.

Concern over impaired reproduction is justified by available experimental evidence. Small test fish exposed to given chemicals over the course of their lifetime have exhibited reproductive impairment ranging from underdeveloped gonads to decreased egg production and abnormal larval offspring. Contaminants administered to adult fish at later stages of their sexual maturation have caused spawning failure or lowered spawning success.

Reproductive impairment in the wild has not been as convincingly established. A few field studies have demonstrated differences in reproductive performance in fish populations from clean waters and those from contaminated waters. Interpretation of such observations is often difficult because of the interplay of variables inherent in natural situations. Food supply, temperature differences, or overall health of the fish may cause temporal and spatial variations in reproductive capacity. To be documented, pollutant effects must be significant enough to be distinguished from these background fluctuations.

The mechanisms by which contaminants can affect fish reproduction are several. Some pollutants are known to influence directly the reproductive cycle of the adult fish: contaminants can alter the synthesis or metabolism of reproductive hormones in fish or interfere with the egg formation process; they may damage reproductive tissues and impair their functions. Pollutants accumulated in adult female fish may be transported to the egg yolk during sexual maturation and result in a direct toxic effect on the developing embryo. An understanding of the mechanisms of reproductive impairment can help determine the most reliable or direct method for gauging the occurrence of the process.

The Coastal and Estuarine Assessment Branch (CEAB) of the Ocean Assessments Division is now funding studies whose intent is to clarify the influence of pollutants on adult fish reproduction in the wild. CEAB's objective is to establish the occurrence of impaired reproduction in fish inhabiting contaminated waters and to develop methods for monitoring this impairment.

To date, CEAB has sponsored research at three laboratories to establish a correlation between pollution and changes in adult teleost fish reproduction. Dr. Jeffrey Cross and his team at the Southern California Coastal Water Research Project have initiated a study on the reproductive capacity of the white croaker (*Genyonemus lineatus*) and the kelp bass (*Paralabrax clathratus*) in polluted Southern California waters. Dr. Robert Spies and his colleagues at the Lawrence Livermore Laboratory are reporting data on the effect of PCBs on the reproduction of starry flounder (*Platicthys stellatus*) from San Francisco Bay. Dr. Donald Malins' laboratory at the National Marine Fisheries Service in Seattle has completed its first year of work on the possible effects of pollutants on the reproduction of English sole (*Parophyrys vetulus*) from Puget Sound.

The three research teams are measuring reproductive success of their experimental species in terms of established parameters: the number of eggs spawned, their fertilization success (eggs fertilized/eggs hatched), their hatching success (eggs hatched/eggs spawned), the percentage of resulting normal larvae, and the percentage of larvae surviving to first feeding. Although these parameters are perforce the best indices of reproduction, their determination can be extremely time-consuming. The researchers are, therefore, also looking for simpler, alternative ways of gauging impaired reproduction. They would like to identify an easily measurable, reliable parameter linked both to pollution levels and to the reproductive capacity of fish. Such a parameter could then be used in a large-scale program to routinely monitor alterations of reproductive processes in the wild. Specific biochemical, physiological, and histological parameters known to be altered by contaminants are being considered as potential monitoring indices.

2. Background

2.1. Laboratory Studies

The three studies now being sponsored by CEAB are building on the limited amount of background knowledge on the interaction of pollutants with fish reproduction derived from laboratory studies, which although they are based on artificial situations, often provide information that is unobtainable in the field. These data can be used to determine the toxicity of specific compounds, to establish threshold levels of their effects, and to establish unequivocal cause-and-effect relationships. Laboratory studies have also provided much of the background material now being used to identify alternative indicators of impaired reproduction.

The Environmental Protection Agency (EPA) has endorsed chronic toxicity tests that are used routinely to determine the sublethal effects of specific industrial compounds on fish (EPA, 1978). These tests require the exposure of a chosen fish species to a determinate contaminant and the assessment of the fish's reproductive success at the end of the exposure period. Newly hatched fry are grown to sexual maturity in water containing a given concentration of contaminant and their eggs and hatchlings are similarly exposed until first feeding. Fertilization success, hatching success, and the survivability of larvae are then determined for fish exposed to different concentrations of the compound. The chronic exposure tests have demonstrated the reproductive toxicity of a number of serious environmental pollutants. However, the concentrations of the pollutants used in the tests are usually much higher than those observed even in contaminated natural waters.

The standard fish used in chronic toxicity studies (EPA, 1978) is the sheepshead minnow (*Cyprinodon variegatus*). Its short lifecycle (four months), the facility with which it grows in aquaria, and the culturing and spawning techniques which have been developed for it make it an advantageous species for the bioassay. Bluntnose minnows (*Pimephales notatus*),fathead minnows (*Pimephales promelas*), and Florida flagfish (*Jordanella floridae*) are now also being adopted as experimental animals. Pollutants can affect reproduction in all four species

(Horning and Neiheisel, 1979; Hedke and Puglisi, 1980; Buckler et al., 1981).

Rowe et al. (1983) used Florida flagfish to gauge the subchronic toxicity of effluent water from a petroleum refinery. The water contained phenols, sulfides, cyanide, and oil. Exposure to 28% concentration of the effluent caused the females' spawning frequency and the number of eggs per spawning to decrease and resulted in more than 50% abnormalities in the hatched fry.

Horning and Neiheisel (1979) grew six-week-old bluntnose minnow fry in water containing copper in concentrations ranging from 18 to 119.4 µg/liter. Differences in reproductive success of the matured fish were related to their exposure to the metal: the number of eggs produced per female minnow consistently fell from an average of 315 in the control to 0 in the fish exposed to the highest concentrations of copper. The gonads of the sterile fish showed immaturity and poor development of testes, and egg resorption and immaturity in ovaries of fish exposed to concentrations of 71.8 and 119.4 µg/liter copper. Poor reproductive success could therefore be clearly correlated to affected gonadal tissues.

Buckler et al. (1981) tested for sublethal effects of mirex and kepone, two heavily chlorinated organic insecticides. The researchers exposed fathead minnows to the insecticides from the time of hatching to sexual maturity, a period of 120 days, and reported evidence of reproductive failure. Kepone in water at concentrations of 0.31 µg/liter caused decreased hatchability of the minnow eggs. Mirex at levels of 3, 13, or 34 µg/liter brought about a decrease in number of spawns and in egg production. However, mirex concentrations of 2, 3, or 7 µg/liter induced production of eggs with increased hatchabilities. These contradictory responses leave doubt about the insecticide's actual sublethal toxicity. On the other hand, the ability of kepone to impair reproduction is strongly suggested by the report.

A chronic exposure study performed by Hermanutz (1978) tested the toxicity of endrin, an organochloride (0.3 µg/liter), and malathion, an organophosphate (24.5, 31.5 µg/liter), on Florida flagfish. Both of these compounds affected the fish's reproductive potential, the first by decreasing the number of eggs produced by the females, the second by reducing the number of fish that matured into reproductive adults.

Jarvinen and Tyo (1978) showed endrin to have a different effect on fathead minnows, demonstrating differences in species sensitivity. These fish were exposed to endrin-contaminated water (0.28 ppb or 0.17 ppb), or were fed endrin-contaminated food (0.28 ppb), or both. Chronic exposure did not alter egg production, but did result in lower survival of contaminated fish as compared to controls, and in strikingly lower survival rates of their offspring. The food and water combination induced the highest number of adult deaths: this result points out the need for taking the food pathway into consideration when doing chronic toxicity studies.

Chronic exposure of fathead minnows to PCBs (DeFoe et al., 1978) showed no influence of the compounds on the reproduction of these fish. Minnows grown in water containing sublethal levels of PCBs (Aroclor 1248, 1260) of up to 3.0 µg/liter showed no change in egg production. Hatching success and larval survival of the fish eggs also remained unchanged. PCBs did affect eggs from control minnows incubated in water containing 2.1 µg/liter Aroclor 1260: the hatching success of the eggs dropped to 27% below control. This suggests acclimation of the fish to PCBs through continual exposure. It also emphasizes the susceptibility of larval stages to contaminants.

Similar acclimation was observed by Hedtke and Puglisi (1980) when they tested for the effect of crankcase waste oil on Florida flagfish. Egg production decreased only after lifelong exposure of the fish to oil concentrations of 3380 µg/liter. Oil affected larvae only when they were produced by unexposed adults and kept in contaminated water after hatching. Oil concentrations as low as 930 μg/liter could then reduce larval survival to about half of that in controls. No comparable effects could be seen among larvae from exposed adults. An adaptation mechanism may thus protect larvae exposed during embryological stages.

Both the protocol and the choice of species used in chronic toxicity tests put limits on its usage. Neither sediment exposure nor food pathway accumulation are taken into consideration when fish are exposed to contaminants dissolved in the water. Effects of compounds with low solubilities (for example, PCBs) can thus be underestimated by the assay. The brief lifespan of minnows and flagfish does not permit an appreciation of the effect of bioaccumulation or of long-term chronic effects of pollutants. Smaller concentrations than those used in the tests might have an effect over a longer time. The hardy nature of the small fish may underestimate the effect of contaminants on more sensitive fish species. Finally, the fish are all freshwater species and may differ from marine organisms in their reaction. Protocols used in the lab therefore fall short of approximating field situations.

2.2. Field studies

A small number of researchers have attempted field studies to gauge the effects of pollutants on fish reproduction in the wild. Such studies are usually less thorough, but more realistic, than laboratory experiments. It is only through them that scientists can gauge the actual threat of a polluted environment to a fish population: a given contaminant's effect must ultimately be assessed in the context in which it is acting.

The protocol in most field studies requires the collection of sexually mature fish from polluted areas. The fish are subsequently induced to spawn in the laboratory, usually via administration of gonadotropin, a hormone which can induce the final stages of ovulation in females. The quantity and viability (percentage fertilization and percentage hatching) of the eggs produced by the fish are then correlated with pollution concentrations in the parental tissues, typically liver or gonads. Alternatively, the histopathological status of parental gonads has been used to measure the fish's reproductive viability. However, this latter alternative is based on untested assumptions of a relationship between histology and reproductive success.

Von Westernhager et al.'s study (1981) offers a demonstration of the type of variability that can hinder the establishment of clear effects of pollutants in the field. The research team collected sexually ripe flounder (*Platicthys flesus*) from different areas of the Baltic Sea. They then sought a relationship between viable hatch of the fish's eggs and the PCB and metal concentrations in their gonads. The workers argued for an effect of PCBs on reproduction: five (out of 59) of the caught flounder had high gonadal PCB content (>100 ppm wet weight) and low viable hatch (<50%). However, about a third of the fish without elevated gonadal PCB levels displayed a similarly low viable hatch.

Freeman and Sangalang (1985) performed a more controlled field study showing how the lowered pH of freshwater bodies could affect fish reproduction. Atlantic salmon (*Salmo salar*) hung in cages in acidic rivers at late stages of sexual maturation had abnormal steroid hormone metabolism, produced fewer eggs, had higher egg mortality, and had less tolerance to cold than fish held in cages in a nearby less acidic river.

McFarlane and Franzin (1978) also showed a pollutant effect on the reproduction of freshwater fish. The scientists compared growth and reproduction of white suckers (*Catostomus commersoni*) from two Canadian lakes, one of which (Hammel Lake) had elevated levels of zinc and cadmium as compared to the other (Thompson Lake). They recorded lower catch per unit effort, lower spawning success, shorter lifespans, and smaller eggs in the suckers from Hammel

Lake than in the ones in Thompson Lake. At the same time, they showed the Hammel suckers to have a faster growth rate and a higher fecundity. McFarlane and Franzin concluded that the fish had developed compensatory responses to counteract the toxic effects of the metal. The researchers failed however to convincingly prove their hypothesis. They did not measure metal body burdens in the fish, nor did they suggest physiological processes that might have led to the observed differences.

Sloof and De Zwart (1983) hypothesized a similar compensatory response for bream (*Abramis brama*) from the Rhine River in the Netherlands. The two investigators measured the growth, fecundity, and mortality of 7,000 bream from clean and polluted waters of Dutch rivers and lakes. Bream from the Rhine River were shown to have a higher mortality, and a higher fecundity, than the ones from the cleaner Lake Braasem.

Sloof and De Zwart's method of measuring fecundity -- estimating the number of eggs in the bream's ovaries and assessing their gonadosomatic index (weight of gonads / weight of fish) -- should be questioned. If it indeed proves to be reliable, their observation of enhanced fecundity in the face of contamination would also indicate a compensatory reaction to pollution. Whether the response is a general phenomenon and how long it might take for it to become established in a population are unanswerable questions at this stage.

Schindler et al. (1985) found no such compensatory adaptation during an eight-year study on the ecosystem of a small Canadian lake. Rather, the scientists clearly demonstrated a decrease in fish population attributable to anthropogenic input. This Canadian team monitored the changes in health, number, and reproductive status of organisms in an experimental lake which they gradually acidified from pH 6.8 to pH 5.0 over the course of eight years. Their control was a similar small lake in the same region, an ideal reference site because of its similar location and ecosystem.

Fathead minnows (*Pimephales promelas*), white suckers (*Catostomus commersoni*), and lake trout were the dominant teleost species in each lake. All three suffered some aspect of reproductive impairment during the study period. Minnows failed to reproduce after the third year of the experiment (pH 5.93), and their short lifespan caused their numbers to decrease to extinction within five years. Lake trout and white suckers continued to spawn regardless of the lake's acidity, but their young-of-the-year populations decreased markedly during the course of the experiment. The environmental pollutants affected the eggs and fry of these fish rather than the adults themselves. For all the fish species, the long-term result was a decrease in population.

3. Potential Indicators of Impaired Reproduction

In an effort to forestall such decreases in populations, scientists are now looking for early warning signs of impaired reproduction. Their research has focused on biochemical, physiological, and histologic aspects of reproductive processes in adult fish. Much of the work has been performed in the laboratory, although important results have occasionally stemmed from field studies.

3.1. Histopathology

Pollutant exposure has been linked with histologically observed changes of the gonadal cycle of fish. Stott et al. (1983) surveyed ovaries from plaice (*Pleuronectes platessa*) collected at the Amoco Cadiz oil spill site. The researchers used the number and types of follicles present in the ovaries to gauge the reproductive capacity of the fish. Gonads from plaice collected at oil-polluted sites at 9, 17, 23, and 27 months after the spill showed distinct differences when compared to control organs. In plaice from the spill site, the follicle maturation cycle in the ovaries had been disrupted, and follicles had not matured at the regular spawning time of the fish. Most of the follicles had "stalled" in the growing stage.

Saxena and Mani (1985) recorded similar histologic observations in the testes of a freshwater teleost, *Channa punctatus*. The fish had been exposed to toxicologically "safe" concentrations of fenitrothion (1.5 ppm), an organophosphate, or carbofuran (5 ppm), a carbamate, for 120 days. Testes of fish exposed to either pesticide showed a decreased gonadosomatic index and a disrupted gonadal cycle. Histologic alterations of the normal reproductive cycle of fish appear therefore to have potential as indicators of pollutant effects on the reproductive process.

Other experiments have shown no influence of contaminants on the gonadal histology of fish. Payne et al. (1978) recorded a lack of histopathologic effects after exposing cunners (*Tautogolabrus adspersus*) to Venezuelan crude oil in a flow-through system for six months. Although the gonadal somatic index of the testes of the exposed cunners decreased, the tissues' histology was not altered. Stott et al. (1980 and 1983) attempted to gauge the effect of petroleum on tissues of fish from the Gulf of Mexico, but they never caught enough fish of a given species to draw substantial conclusions. The histology of the gonads of the fish they did catch did not show significant alterations.

3.2. Reproductive Hormone Synthesis

More drastic histologic changes in fish gonads caused by pollution have repeatedly been associated with impaired synthesis of reproductive hormones. The effect of such impairment on the reproductive process still needs to be defined. If a relationship can be established, histologic observations linked with measurement of the hormone-synthetizing abilities of gonadal tissues could have potential as indices of impaired reproduction.

Sangalang and Freeman (1974) recorded the effects of cadmium on tissue development and hormonal production in the testes of brook trout (*Salvelinus fontinalis*). Ten days' exposure of the fish to 1 ppb cadmium in water resulted in vascular enlargement, congested blood vessels, and regression of tissues in the testes of the fish. Concomitantly, blood levels of steroid hormones recorded over the reproductive season of the trout were markedly different in contaminated and control fish. Sangalang and Freeman suggested that this disparity may be correlated with the metal's effects on the tissues. They hypothesized cadmium to be inducing early testicular regression, and thus a shortening of the spawning season.

A similar paper by Sangalang and O'Hallaran (1972) reported extensive hemorrhagic necrosis in testes of brook trout exposed to 25 ppb cadmium in water. The testes' ability to synthesize steroid hormones in vitro had also been affected by the cadmium: the gonads were unable to produce testosterone or 11-ketotestosterone from precursors. Ultimate effects of this damage on the reproductive cycle of the fish remained untested.

Histological damage concomitant with altered steroid synthesis also resulted from exposure of Atlantic cod (*Gadus morhua*) to PCBs (Freeman et al., 1972). Cod fed PCB-contaminated food (1-50 µg/g for 5-1/2 months) showed breakdown and inhibition of testicular spermatogenic tissues (at 1, 5, 10 µg/g) and disintegration and degeneration of spermatogenic elements (at 25, 50 µg/g). Concurrent with these changes, the hormone-synthesizing ability of the testes became impaired.

Sivarajah et al. (1978) could show no such effects of PCBs on the testes of rainbow trout and carp. After injecting the compounds into the fish (25 mg/kg body weight) once a week for four weeks, they found only slight changes in the histopathology of the gonads: the testes were unaffected except for some damaged spermatozoa; the ovaries contained eggs with fragmented cytoplasms, reflecting proliferation of the smooth endoplasmic reticulum. The researchers attributed these limited changes to a decrease in androgen levels seen in the circulating blood. They also therefore associated histological effects with endocrine changes.

3.3. Vitellogenin

Pollutant-induced changes in endocrine levels might be reflected by alterations of related parameters. The protein vitellogenin, an inherent component of fish egg yolk, is produced by the liver when induced by estrogen. Decreased production of vitellogenin could result in reduced egg formation. Chen and Sonstegard (1984) recently showed that pollutants could alter blood levels of vitellogenin, and hence suggested that levels of the protein could be used to monitor fish fecundity. The scientists fed mirex (0.5,5,50 ppm) or PCB (3, 30, 300 ppm) or a mixture of both (30 ppm PCB + 5 ppm mirex) to rainbow trout for six months. They then injected the fish with estradiol and measured the resulting levels of vitellogenin in the blood serum. Vitellogenin production was greatly reduced in fish fed contaminated food. Whether other pollutants would cause similar declines and whether these declines could result in lowered egg production are questions that still need to be answered by the researchers.

3.4. Mixed Function Oxidases

Mixed function oxidases, MFOs, are enzymes involved in both xenobiotic and steroid metabolism. Hepatic MFO activity can be induced by pollutants and is considered an index of environmental stress. Spies et al. (1985) conducted the first field study to correlate impaired reproductive capacity in feral fish with contaminant-induced alteration of MFO activity. This parameter is now being considered as a potential monitoring index.

Spies' study, funded by CEAB, aimed to determine whether organochloride pollutants from San Francisco Bay were affecting the reproduction of the resident starry flounder (*Platicthys stellatus*). In the study, starry flounder collected from different areas of the Bay were spawned in the laboratory. Their reproductive success was then determined in terms of percentage hatching and percentage fertilization of the spawned eggs, embryological success, and percentage larval abnormalities. The activity of a mixed function oxidase in the liver of the flounder was measured concomitantly with these reproductive parameters.

Spies' results showed that the levels of MFO activity in the flounder reflected the degree of pollution of the sediments at their collection sites. More importantly, Spies demonstrated the existence of an inverse correlation between egg fertilization success and hepatic MFO activity in the female parent: fish with highly induced MFOs had the lowest fertilization rate. Spies' evidence has revealed MFO levels as an attractive potential monitoring index.

3.5. Steroid Hormone Blood Levels

Spies proposed three plausible hypotheses to explain the correlation between MFO activities and reproductive success he observed in the flounder: (i) that excessive contaminant metabolites formed by the induced MFOs accumulate in the eggs and prevent successful fertilization; (ii) that the parent compounds increase MFO levels and reduce fertilization success by two unrelated but proportional mechanisms; (iii) that activation of MFOs results in increased steroid hormone metabolism, with subsequent repercussions on reproduction. Spies' third hypothesis is receiving increasing attention and is thus far supported consistently by a small but growing number of studies. It is known that steroid-metabolizing MFOs of different fish species can be induced by pollutants (Hansson et al., 1980; Forlin and Hansson, 1982; Truscott et al., 1983). The effect of increased MFO activity on the fish is still unclear. Some experiments suggest that increased production of hormonal metabolites might lead to decreased blood hormone levels, and thus to impaired reproductive processes; supporting evidence is still tenuous.

Hansson et al. (1980) demonstrated that both Clophen A-50 (a PCB) and 3-methylcholanthrene (a polynuclear aromatic hydrocarbon) could induce the activity of androstenedione-hydroxylating enzymes in the livers of rainbow trout (*Salmo gairdneri*). Androstenedione is a main precursor to male and female reproductive hormones (Makin, 1985). Forlin and Hansson (1982) showed that treated municipal wastewater could similarly alter the activity of specific steroid-metabolizing enzymes in the trout. Neither study connected the altered enzymatic activity with possible changes in the level of hormones in the fish's blood. Conversely, Truscott et al. (1983) observed a general decrease in testosterone levels in the blood of landlocked salmon (*Salmo salar sepago*) and winter flounder (*Pseudopleuronectes americanus*) exposed to crude oil, but did not look for corresponding changes in steroid-metabolizing hepatic enzymes.

A study monitoring both hormone levels and enzyme activity in fish was performed by Sivarajah et al. (1978). The workers injected trout and carp with PCBs once a week for four weeks (25 mg/kg) and periodically collected blood and liver samples from the fish. After four weeks of treatment, the plasma levels of androgens, oestrogens, and corticosteroids of both fish species had decreased. Concomitantly, hepatic MFO activities had become induced. Sivarajah et al. concluded that the administration of PCBs to the fish had resulted in the decreased hormone levels through the induction of the steroid-metabolizing enzymes. Whether the observed decrease could affect reproduction, however, is unknown.

3.6. Contaminant Concentrations in Eggs

Spies' field study of flounder also demonstrated an inverse correlation between embryological success and chlorinated hydrocarbon concentrations in the fish's eggs (Spies et al., 1985). This relationship suggests a direct toxic effect of contaminants on early reproductive stages, hence the repercussion on the offspring of adults exposed to pollutants.

Monod (1985) demonstrated an increased mortality of Lake Geneva charr (*Salvelinus alpinus L.*) embryos related to the lipid-normalized PCB and DDT concentration in the fish's eggs. Eggs containing 78 ppm PCB per lipid weight showed a mortality of 76%. Lower mortality rates were more common, but the correlation observed by Monod was significant, with $P<0.01$. Monod suggested that contaminants have a hazardous effect on the charr in Lake Geneva, but did not directly inculpate PCBs or DDT. Other pollutants, not measured in Monod's experiment, are also present in the lake.

A number of earlier studies by Macek (1968), Johnson and Pecor (1969), and Burdick et al. (1964) had also reported that high concentrations of DDT accumulated in fish eggs had induced death of their fry. Egg contaminant levels therefore seem to have potential as alternative indices of impaired reproduction. It is unlikely however that critical levels established for a pollutant will be applicable across fish species.

4. Ongoing Efforts

All of the parameters discussed above are far from perfected viable indicators of impaired reproduction. Three projects now being sponsored by CEAB are developing the viability of the parameters while providing understanding of the interaction between contamination and fish reproduction. The studies will accrue information which will contribute to the establishment of a monitoring program for fish reproductive success.

4.1. Spies et al.

Spies et al. are now gauging the reproductive viability of San Francisco Bay starry flounder in the laboratory as well as in the field (Spies, 1985). In 1986, the research team will initiate a laboratory study to test the effect of long-term exposure to specific environmental pollutants on the reproductive viability of flounder. Benzo(a)pyrene and Aroclor 1260 will be fed to female flounder at levels approximating those found in San Francisco Bay sediments. The feeding period, 90 days, will include part of the sole's reproductive cycle. The fish will subsequently be spawned and reproductive parameters measured. Levels of reproductive hormones in the fish's blood will be measured concomitantly with MFO activity.

In the field, Spies will determine more specifically the degree of reproductive impairment of fish from different parts of the bay and look for evidence of complete spawning inhibition in the most polluted areas. By increasing the number of sampling sites, Spies intends to obtain flounders exposed to a range of pollutant concentrations. He wishes to demonstrate that this range is reflected by the MFO levels and reproductive capacity of the fish.

Flounder will be collected in the wild and subsequently spawned in the lab, as done in the past. However, photoperiod manipulation will be used in 1986 to mimick the inception of the flounder's reproductive season and hence to induce spawning. This technique is preferable to gonadotropin injections -- used in the 1985 study -- since hormonal administrations may result in spawns that would not otherwise occur, and hence cause "false positive" results.

4.2. Malins et al.

Spies' work on potential reproductive parameters in starry flounder is now being paralleled by another CEAB-sponsored effort. Malins and his collaborators at the National Marine Fisheries Service Laboratory in Seattle have initiated two studies to explore the effects of pollutants on the reproductive capacity of English sole (*Platichthys flesus*) from Puget Sound (Malins et al., 1985). In a field study, they are measuring the reproductive success of soles collected at two spawning sites in Puget Sound. In a complementary laboratory study, the researchers are administering sediment extracts to gravid female sole and gauging the subsequent reproductive success of the fish. Both field and laboratory studies use fertilization success (eggs fertilized / eggs spawned) and hatchability (eggs successfully hatched / eggs fertilized) to determine the reproductive viability of the sole's eggs.

Malins' research, like Spies', is also aimed at identifying an easily measurable parameter linking pollution and reproductive success. Potential indicators of contamination are being measured in the study fish in conjunction with reproductive parameters. Malins hopes to use these indicators to demonstrate a gradient in reproductive success reflecting the pollution levels of Puget Sound. Biochemical indices include the activities of MFOs in the liver and the levels of selected antioxidants -- glutathione and vitamin C -- in the liver and gonads of the fish. Chosen chemical parameters are liver levels of PCBs and bile content of aromatic hydrocarbon metabolites. Collected fish are also being examined for the presence of liver lesions, especially neoplasms. These histopathologic conditions are becoming convincingly correlated with pollution exposure.

Tentative results from the first year's fieldwork found only one of the above parameters to be linked to both reproduction and pollution: vitamin C concentrations in the gonads of the female sole were positively correlated with both fertilization success and aromatic hydrocarbon metabolite concentrations in the bile, but negatively correlated with liver PCB concentrations. The significance of these relationships is unclear. The role of vitamin C in reproduction and detoxification is poorly understood; high vitamin C levels in the diet have been associated with increased fertilization success in salmon (Sandnes et al., 1985), and liver reserves of vitamin C in striped mullet have been shown to decrease on long-term exposure of the fish to crude oil (Thomas and Neff, 1984). Malins' results must now be assessed within a framework of such disparate knowledge.

Histopathologic examination of the fish offered another interesting observation. The prevalence of liver neoplasms in female sole caught at two spawning sites was considerably lower than in females from the Duwamish River, a highly polluted nonspawning area of Puget Sound (4% and 0% prevalence at spawning sites vs.17% in the Duwamish River). Hepatic neoplasm prevalence in male sole was about 15% at all sites. The researchers suggest that female sole affected by neoplasms may not be participating in the spawning process. This hypothesis is now being tested by further sampling -- at clean as well as polluted sites and during spawning and nonspawning seasons -- and by attempting to spawn females collected at the Duwamish River.

Preliminary laboratory results on the effect of contaminants on sole reproduction are less ambiguous. Sole injected 1-4 days before spawning with Eagle Harbor sediment extracts (resulting in a dosage of 6.8 mg aromatic hydrocarbons/kg body weight) produced eggs with decreased fertilization success and decreased percentage of viable hatch. Four out of the eleven treated fish did not spawn at all; these four fish showed higher hepatic MFO activity than did spawning fish. Malins and his coworkers therefore suggest that "a relationship may exist between MFO activity and inhibition of spawning" (Malins et al., 1985). Duwamish River extracts injected in prespawning English sole (resulting in body burdens of 0.52 mg aromatic hydrocarbons/kg and 0.04 mg PCBs /kg) did not affect the sole's reproduction, but did induce the hepatic MFO activites of the fish. The detrimental effects of contaminated sediments on sole reproduction were also demonstrated by an ancillary laboratory experiment. Gravid female fish were injected with Duwamish River sediment extract for twelve days prior to their predicted spawning time. Four of eight sole thus treated did not spawn. These experiments provide evidence for the reproductive toxicity of some Puget Sound sediment.

During 1986, their second year of work, Malins and his collaborators will repeat their field and laboratory experiments to corroborate their initial results. In addition, they will increase sampling of sole at reference and spawning sites to clarify the relationship between the presence of neoplastic lesions and spawning. Further fieldwork will assess the ovaries of the female sole at regular intervals throughout the spawning cycle to help determine whether female sole in contaminated areas undergo normal ovarian development. Laboratory work will test the effect of contaminants on the spawning performance of male as well as female fish and on the quality and viability of the resulting sperm.

4.3 Cross et al.

A third CEAB-funded study on reproduction in feral fish has been initiated recently by Dr. Jeffrey Cross and his collaborators at the Southern California Coastal Waters Research Project (Cross, 1985). This research team is monitoring the reproduction of white croaker and kelp bass from clean and polluted Southern California waters. White Point off the Palos Verdes Peninsula, the selected contaminated sampling site, receives large amounts of municipal wastewater discharges, and its sediments contain high concentrations of PCBs and DDTs. Fish will be collected from this site and from a reference site, and will be spawned in the laboratory.

Cross and his coworkers will use the standard reproductive parameters to gauge the reproductive success of the fish. In addition, they will look for cytogenetic alterations in fish embryos. They will attempt to correlate these parameters with contaminant concentrations in fish tissues, MFO activities in their livers, and levels of endocrine hormones. Because the last two are known to fluctuate over the yearly reproductive cycle of the fish, Cross et al. will be measuring them monthly or bimonthly so as to obtain their range of variation during the course of a year.

Cross and his coworkers will also be looking for any histologic changes in the fish's reproductive organs and for possible alterations of their reproductive cycle. In an earlier histologic study, Cross et al. (1984) had observed high levels of atresia -- reabsorption of mature eggs -- in ovaries of fish from polluted sites off the Southern California coast. Because they were unable to find a comparable clean control site, the scientists could not determine whether the observed atresia was a natural or a contaminant-induced phenomenon. In 1986, Cross intends to determine whether atresia can indeed be related to pollutant effects.

Cross et al. have completed an exhaustive literature search on the effects of contaminants on teleost reproduction. The research group included in their review physiological, biochemical, cytologic, and teratogenic effects, and uncovered no more than eighty references. A paucity of information is evident in this field; resource managers are still far from being able to assess whether pollutants can ultimately affect fish populations.

5. Summary Tables

The following two tables summarize the main features of the studies reviewed in this report. They especially emphasize the physiological changes in the fish indicating reproductive impairment. Table 1 (three pages) is dedicated to laboratory studies; table 2 condenses the fieldwork.

Table 1. Laboratory studies on the effect of pollutants on fish reproduction

Reference	Fish Species	Contaminant	Concentration	Indicator of Reproductive Impairment	Other Potential Indicators
Buckler et al.,1981	*Pimephales promelas* (fathead minnow)	mirex kepone	2-34 ug/l 0.31 ug/l	decresed number of spawns, egg production, decreased egg hatchability	
Chen and Sonstegard, 1984	*Salmo gardnieri* (rainbow trout)	mirex PCB mirex/PCB	0.5,5,50ppm 3,30,300ppm 30/5 ppm		reduced vitellogenin levels in blood
DeFoe et al., 1978	*Pimephales promelas*	Aroclor 1248 and 1260	≤3 ug/l	decreased hatching success	
Freeman et al., 1982	*Gadus morhua* (Atlantic cod)	PCB	1-50 ug/l		breakdown and inhibition of spermatogenic tissue, impairment of hormone synthesis
Forlin and Hansson, 1982	*Salmo gardnieri*	treated municipal wastewater			altered activity of steroid-metabolizing enzymes
Hansson et al., 1982	*Salmo gardnieri*	clophen A-50 3-methylcholanthrene			induction of steroid-hydroxylating enzymes
Hedke and Puglisi, 1980	*Jordanella floridae* (Florida flatfish)	crankcase wasteoil	930 and 3380 ug/l	reduced larval survival	
Hermanutz, 1978	*Jordanella floridae*	endrin malathion	0.3 ug/l 24.5 and 31.5 ug/l	decreased egg production, reduced larval survival	

Table 1. Continued

Reference	Fish Species	Contaminant	Concentration	Indicator of Reproductive Impairment	Other Potential Indicators
Horning and Neiheisel, 1979	*Pimephales notatus* (bluntnose minnow)	copper	18-119.4 ug/l	decreased egg production	immature and poorly developed gonads
Jarvinen and Tyo, 1978	*Pimephales promelas*	endrin	0.17 and 0.28 ppb	lowered survival of contaminated fish and their offspring	
Malins et al.,1985	*Parophyrys vetulus* (English sole)	sediment extracts	6.8 ppm PAH	decreased fertilization success, inhibition of spawning	
Payne et al., 1978	*Tautogolabrus adspersus* (cunner)	crude oil			decreased gonadosomatic index
Rowe et al., 1983	*Jordanella floridae*	effluent from petroleum refinery	28%	reduced spawning, high percentage of abnormalities among fry	
Sangalang and Freeman, 1974	*Salvelinus fontinalis* (brook trout)	cadmium	1 ppb		histological abnormalities of testes, changes in hormone levels in blood
Sangalang and O'Halloran, 1972	*Salvelinus fontinalis*	cadmium	25 ppb		histological changes in testes, changes hormone levels in blood,changes in steroid synthesizing ability
Saxena and Mani, 1985	*Channa punctatus*	fenitrothion carbofuran	1.5 ppm 5 ppm		decreased gonadosomatic index, disrupted gonadal cycle

Table 1. Continued

Reference	Fish Species	Contaminant	Concentration	Indicator of Reproductive Impairment	Other Potential Indicators
Sivarajah et al., 1978	*Salmo gairdneri*, *Cyprinus carpio* (carp)	Aroclor 1254	25 ppm (body weight)		damaged spermatozoa, eggs with fragmented cytoplasm, decreased levels of androgen in blood
Truscott, et al., 1983	*Salmo salar* (Atlantic salmon), *Pseudopleuronectes americanus* (winter flounder)	crude oil			decreased testosterone levels

Table 2. Field studies on the effect of pollutants on fish reproduction

Reference	Fish Species	Contaminant	Study site	Indicator of Reproductive Impairment	Other Potential Indicators
Freeman and Sangalang, 1985	*Salmo salar*	lowered pH	acidified river	reduced egg production, increased egg mortality	abnormal steroid metabolism
McFarlane and Franzin, 1978	*Catostomus commersoni* (white sucker)	heavy metals	Hammel Lake, Canada	lowered catch per unit effort, lowered spawning success, small eggs	
Monod, 1985	*Salvelinus alpinus* (charr)	PCB, DDT	Lake Geneva Switzerland	increased egg mortality	
Schindler et al.,1985	*Pimephales promelas*, *Catostomus commersoni*, *Salmo trutta* (brook trout)	lowered pH	experimental lake in Canada	decreased recruitment, impeded reproduction	
Sloof and De Zwart, 1983	*Abramis brama* (bream)	organo-chlorines, PAHs, aromatic amines	Rhine River, the Netherlands	increased mortality, decreased gonadosamatic index	
Spies et al., 1985	*Platichthys stellatus* (starry flounder)	PCB	San Francisco Bay	decreased egg fertilization	increased MFO activity
Stott et al., 1983	*Pleuronectes platessa* (plaice)	crude oil	Amoco Cadiz oil spill site		disrupted gonadal cycle
von Westerhagen et al., 1981	*Platichthys flesus* (Baltic flounder)	PCB, heavy metals	Baltic Sea	decreased viable hatch	

6. References

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